Plate Reader measurements for *B.subtilis*

This protocol was 96 well plates (black walls, clear bottom; Greiner Bio-One, Frickenhausen, Germany) and the plate reader *Synergy2* (BioTek).

- Start dayculture (not later than 10 am) by preparing 10 ml fresh-made, **pre-heated** MCSE w/o antibiotics and dilute your overnight culture 1:500 (= 20 µl in 10 ml). Put at 37°C and let shake until OD 0,2. This takes approx. 4-5 hrs.
- Start preparing the 96 well plate by preparing a reasonable pipetting scheme. Fill the outer wells with 100 µl of water since this prevents evaporation. Do not forget a blank (MCSE) for OD correction. **Be aware not to touch the lid or bottom of the plate with your fingers!**

- When the **first** dayculture has reached OD 0,2 (even though others made need a little longer) dilute all cultures to an OD **0,05 in a final vol. of 200 µl [0,01 if LB]** in a 1.5 ml tube. Let them shake until you have calculated everything (vol. of culture, vol. of MCSE) and provided the tubes with the correct vol. of MCSE.
    - **Here, you have to work really quickly since the cells are still shaking and growing and the longer you need for calculating and pipetting the more incorrect you are in the end!**
- vortex the tubes shortly and pipet **100 µl per well** according to your pipetting scheme.
- Start the luminescence assay: Grow cultures for 18 hours under 37°C and agitation. The growth is measured every 10 minutes at a wavelength of 600nm (OD_{600}). The luminescence is measured every 10 minutes

Protocol generously provided by the lab
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